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Apr. 2000

# On-site bioremediation of oily slu contaminated soil in tank farm ar at BPCL Refinery, Mumbai

*Prepared for* **Bharat Petroleum Corporation Ltd.**
*Mahul, Mumbai*

T.E.R.I. Project Report No. 1999BM65

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A suggested format for citing this report is as follows.
T E R I. 2000
On-site bioremediation of oily sludge contaminated soil in tank farm area at BPCL Refinery, Mumbai
New Delhi: Tata Energy Research Institute. 20 pp.
[T E R I Project Report No. 1999BM65]

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Duration of project : Six months

Date of commencement : 15.10.1999

Date of completion : 14.04.2000

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# Acknowledgements

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This project entitled "On-site bioremediation of oily sludge contaminated soil in tank farm area at BPCL Refinery, Mumbai" was a collaborative project between Tata Energy Research Institute, New Delhi and Bharact Petroleum Corporation Ltd. Refinery, Mumbai. The team members of this project gratefully acknowledge Mr M B Lal, Director, BPCL Refinery, Mumbai for his encouragement and support to implement this technology at BPCL Refinery for oily sludge management. We offer our thanks to Mr S K Phull, Executive Director, BPCL Refinery, Mumbai for ensuring continuous support during the course of this work.

We are indebted to Mr T Lal, DGM - Technology Department and Mr C J Iyer, Chief Manager for initiating this project at BPCL Refinery.

Our thanks are due to Mr Atul D Vyawahare, Manager (Environment Control), Mr P D Amin, Manager (Energy & Environment Co-ordination) and Mr R Ganesh, Manager (Energy Conservation) at Technology Department for their continuous support and valuable suggestions extended during the course of this project.

We gratefully acknowledge Dr Himmat Singh, Advisor (R&D), BPCL Refinery Mumbai, Mr P A Dandekar, DGM (Special Projects) and Dr G P Rai, Sr Manager (R&D), BPCL Refinery for their constructive suggestions and co-operation extended to us at various stages of this work.

We are also grateful to Dr R K Pachauri, Director, TERI for motivating us to carry out this project. We are also thankful to Dr Vibha Dhawan, Dean, Bioresources & Biotechnology for constant support and encouragement to carry out the present project in collaboration with BPCL Refinery Mumbai. Finally, we appreciate the efficient administrative support provided by Mr G Gopalakrishnan.

(Banwari Lal, Jeevan Jyot, Sanjeet Mishra, Dhruva Bhattacharya, Vinod Kumar)

# 1

# Introduction

Petroleum refineries occupy a key role in the economy of our country. Presently, there are 14 oil refineries of various sizes operating in India. These oil refineries generate huge volume of oily sludge. Management of oily sludge is one of the major problems being faced by the refineries.

Oily sludge is hazardous waste that causes enormous environmental pollution. According to the existing classification of hazardous waste, the refinery oily sludge falls under Hazardous Waste Category no. 10 and 12. Due to stringent norms by regulatory authority and corporate responsibility of oily refinery to protect environment, new techniques are continuously in demand in India to manage oily sludge. However, in developed countries, new technologies are continuously being invented and implemented for treatment of oily sludge. Several technologies, which were considered emerging a few years back are now well known and accepted in the field of hazardous waste treatment. One such technology is the bioremediation of oily sludge using specialised bacteria. These specialised bacteria have the capability to breakdown and digest oily sludge and convert them into harmless products like carbon dioxide, water and fatty acids.

## What is bioremediation

Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to non-toxic compounds. There are innumerable strains of microbes under basic categories of bacteria, yeast or fungi, which bioremediate oily sludge through digestion of harmful chemicals and compounds present in oily sludge into simpler, less toxic or non-toxic substances. Microorganisms, like all living organisms, need nutrients (nitrogen, phosphate and trace metals), carbon and energy to survive. These beneficial microbes break down a wide variety of organic (carbon-containing) compounds found in oily sludge to obtain energy for their growth. Many species of soil bacteria, use petroleum hydrocarbons as a food/energy source, transforming them into harmless substances consisting mainly of carbon dioxide, water and fatty acids. Bioremediation harnesses this natural process by promoting the growth of microbes that can effectively bioremediate specific contaminants and convert them to non-toxic by-products. There are two basic types of bioremediation. "Biostimulation" provides nutrients to the indigenous microbial populations. This promotes growth and increases metabolic activity, which is used to bioremediate contaminants. "Bioaugmentation" introduces specific blends of microorganisms into a contaminated environment to initiate the bioremediation process.

As a timely response to the increasing importance of such microorganisms that efficiently bioremediate and detoxify oily sludge, TERI has developed a bacterial consortium known as Oilzapper. Oilzapper is a mixture of five natural occurring bacterial strains, which can bioremediate all the fractions of oily sludge at very fast rate. Oilzapper is being used for bioremediation of oily sludge by Indian Oil Corporation Ltd., Bharat Petroleum Corporation Ltd., Hindustan Petroleum Corporation Ltd and Oil India Ltd.

## *Advantages of Bioremediation*

- It is an ecologically sound, natural process; sludge eating bacteria can increase in numbers when an oily sludge (contaminant) is available. When the contaminant is bioremediated, the microbial population naturally declines. The residues from the biological treatment are usually harmless products (carbon dioxide, water and fatty acids).
- Instead of merely transferring contaminants from one environmental medium to another (e.g., from water to air or land) bioremediation destroys the target chemicals.
- Bioremediation is usually less expensive than other technologies that are often used to clean up hazardous waste.
- Bioremediation can often be accomplished *in situ*. This eliminates the need to transfer large quantities of contaminated waste and the potential threats to human health and the environment that can arise during transportation.

# 2 Methods

## Mass scale production of oily sludge degrading bacteria (Oilzapper) and their application at tank dyke area of tank 115

"*Oilzapper*" (a sludge degrading bacterial system) was grown in a fermenter on minimal salt medium. After 16 hours of growth cultures were harvested and mixed with a carrier material. The carrier based Oilzapper (containing $10^{10}$ cells/g carrier material) was filled in sterilized poly bags (Figure 1). Poly bags containing Oilzapper were sealed and kept in dark at 25°C for 7 days for curing.

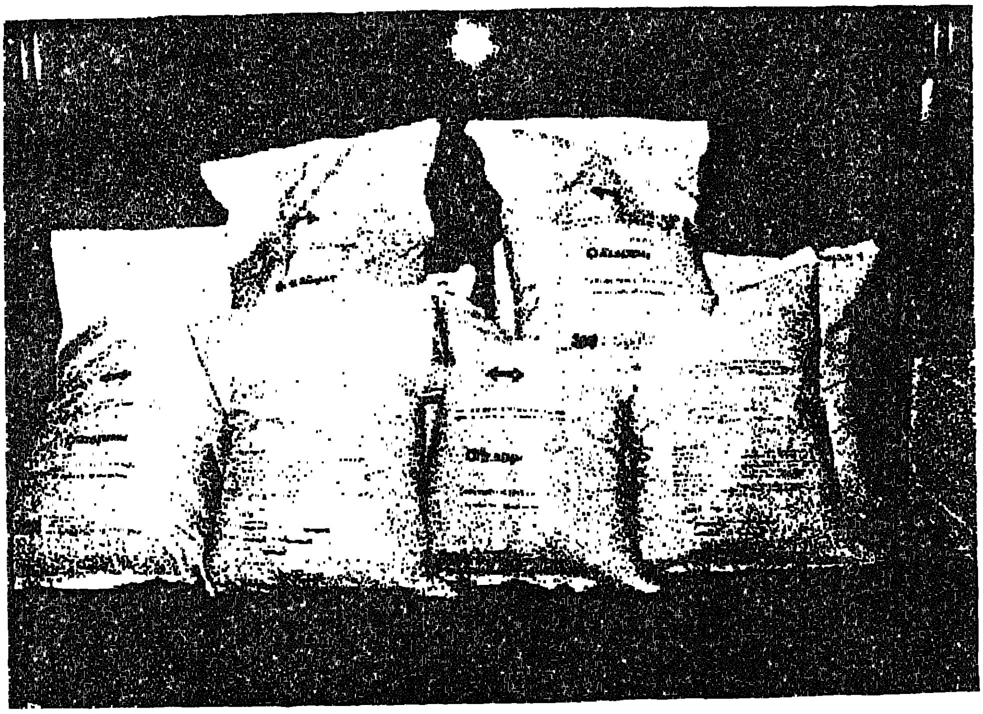


**Figure 1**. Oilzapper - A carrier based crude oil/oily sludge degrading bacterial consortium

Cured culture packets were transported to BPCL Refinery, Mumbai and applied in experimental sludge treatment site.

Oily sludge was distributed heterogeneously in experimental site (tank dyke area of tank 115), therefore, the site was divided into three blocks. *"Oilzapper"* and nutrients were applied in experimental blocks B and D. In block C, only nutrients were applied. Soil in block B, C and D were tilled for uniform distribution of bacterial cells.

## Selection of site and experimental design for bioremediation of oily sludge

Experimental site for *in situ* and on site bioremediation was selected in Tank Dyke of crude tank 115 at BPCL Refinery, Mahul, Mumbai (Figure 2). The land in tank dyke was contaminated with tank bottom sludge. Oily sludge in tank dyke was distributed heterogeneously and some spots in tank dyke were saturated with sludge. Oily sludge was mixed in soil of tank dyke by tilling of soil. After mixing of oily sludge in soil, the level of oily sludge contamination in soil at zero time (just before experiment was initiated) was estimated. Initially, the dyke area was divided into four blocks. However, during cleaning of dyke in the month of December, 1999 the sludge from block A was manually removed and dumped into sludge pit. Therefore, the block A was excluded from treatment plot. The blocks B, C and D were sampled at 10 points to a depth of 30 cm before the experiment was initiated and after 60 days of initiation of bioremediation work. Oily sludge was heterogeneously distributed in the soil samples collected from tank dyke, therefore, the soil was powdered by soil pulverizer and mixed uniformly by passing through a sieve to uniformly distribute sludge. Oily sludge from 10 g soil samples collected at zero time (just before initiation of work) was extracted with hexane, methylene chloride and chloroform (100 ml each) consecutively (93-95% extraction efficiency). After extraction all three extracts (obtained by hexane, methylene chloride and chloroform) were pooled and dried at room temperature by evaporation of solvents under gentle nitrogen stream in fume hood. After solvent evaporation, the amount of sludge recovered from 10 g soil was determined by simple gravimetry. The blocks in tank dyke were also sampled at 2 months, 4 months and 6 months intervals. The sampling and extraction of oily sludge from soil samples collected after 2 months, 4 months and 6 months of initiation of work were identical to zero time samples and the amount of total petroleum hydrocarbon (TPH) recovered from soil sample collected after 2 months, 4 months and 6 months were quantified by gravimetry.

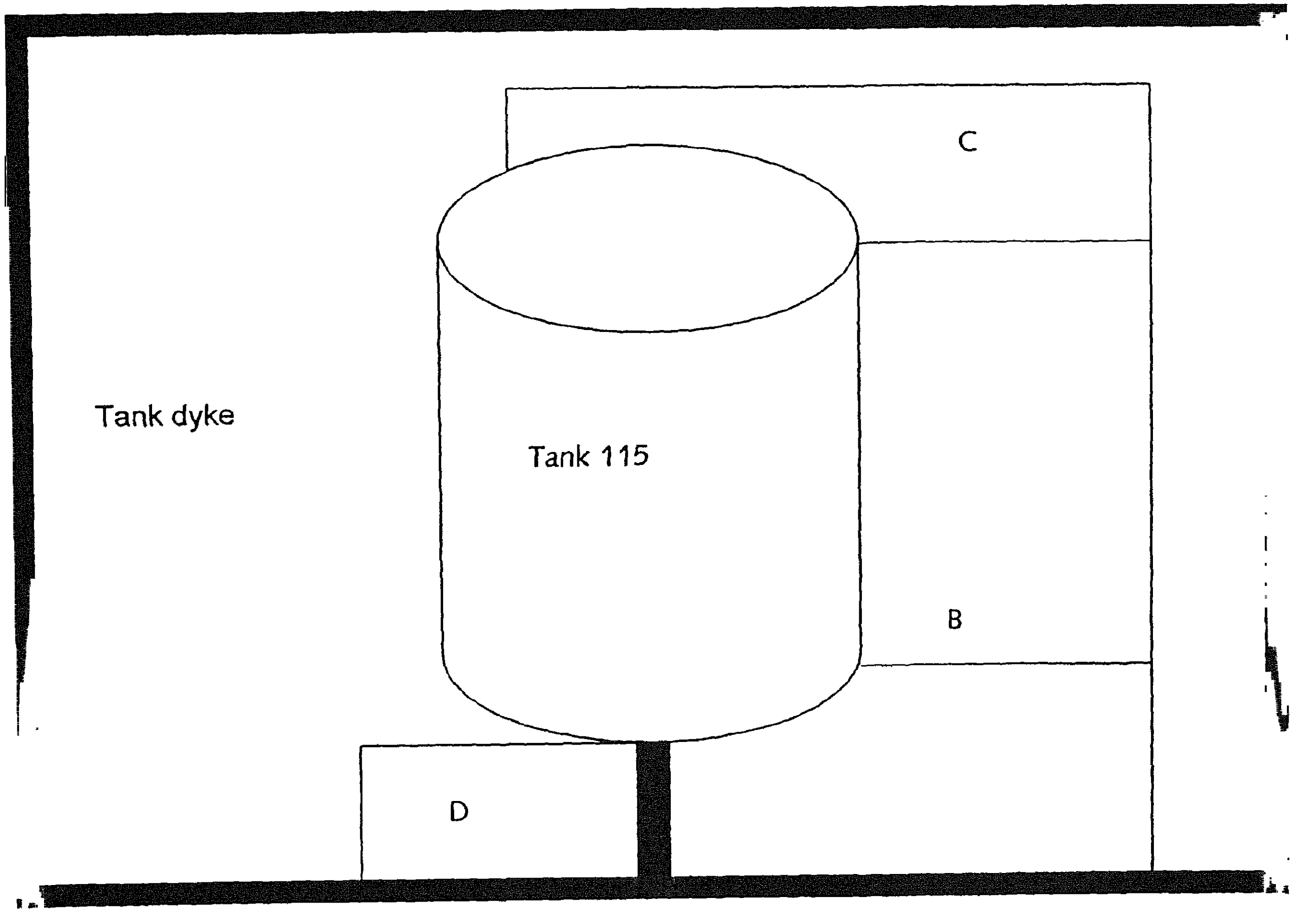


**Figure 2.** Layout of bioremediation site at tank farm area of tank 115

Soil samples from bioremediation site collected at zero time were analyzed for soil bulk density, water holding capacity, organic carbon, nitrogen, available phosphorus, potassium, moisture and pH to see the nutrient status of soil. The soil samples of treatment site would also be analysed after completion of bioremediation work to see the improvement in soil conditions.

## Oily sludge analysis

Oily sludge that was lying in tank dyke of tank 115 was analysed before initiation of work. The total petroleum hydrocarbon (TPH) was extracted from oily sludge using hexane, methylene chloride and chloroform. After evaporation of solvents the amount of TPH recovered from 10g oily sludge was quantified. The TPH that was extracted from oily sludge was further fractionated into alkane, aromatic, asphaltanes and NSO fractions (Nitrogen, Oxygen, and sulphur containing compound) with a silica gel column. The ash content of oily sludge was estimated after extraction of TPH from oily sludge. A known amount of oily sludge was taken in crucible and heated at 600°C for five hours. After cooling the amount of ash recovered from 10g sludge was quantified by gravimetry.

## Composition of TPH recovered from oily sludge

The TPH recovered from oily sludge at zero time and after 2, 4 and 6 months were fractionated into alkane, aromatic asphaltane and NSO (nitrogen, oxygen, and sulphur containing compound) with a silica-gel column. The TPH was first dissolved in n-pentane and separated into soluble and insoluble fractions (asphaltanes). The soluble fraction was loaded on silica-gel column and eluted with different solvents. The alkane fraction was eluted with hexane, followed by the aromatic fraction that was eluted with benzene. The NSO fraction was eluted with chloroform and methanol. Alkane and aromatic fractions were concentrated by evaporation of solvents and then 0.2 µl of each was analyzed by gas-liquid chromatography (GLC Hewlett Packard, 5890 Series II) to identify all the compounds present in the alkane and aromatic fractions. In the alkane fraction, various alkane compounds were identified by matching the retention time with authentic standards. Simultaneously, the identification of each alkane compound was also confirmed by spiking the alkane fraction. A known amount of each standard alkane compound was added to the alkane fraction and injected into GLC (co-injection) and the peak area of each alkane compound increased as a result. Similarly, the polycyclic aromatic hydrocarbon compounds were identified by matching the retention time with an authentic standard and confirmed by spiking the sample with authentic standards.

# 3

# Results

## Analysis of soil at experimental site

Before initiation of bioremediation work at BPCL Refinery, Mumbai, the soil of 30 cm horizon (surface to 30 cm depth) was collected from the tank dyke of tank 115 using soil sampler. Zero time soil analysis of experimental site showed 4.8% organic carbon, 62 ppm available phosphorus and 175 ppm potassium, 0.08% total nitrogen, 52% water holding capacity, pH of 7.77 and soil bulk density of 0.99g/cm$^3$ with black as the soil texture (Table 1).

After completion of bioremediation of oily sludge in tank dyke of tank 115, the soil characteristics at bioremediation site has improved. At zero time, the organic carbon in soil of bioremediation site was high (4.8%) due to oily sludge contamination in soil. After bioremediation of oily sludge, the organic carbon in soil reduced from 4.8% to 2.6%. The total nitrogen in soil at bioremediation site was increased from 0.08% to 0.09% (Table 1). At zero time, the water holding capacity and bulk density of soil at bioremediation site was less as compared to normal agricultural land due to oily sludge contamination in soil. After bioremediation of oily sludge, the water holding capacity and soil bulk density has improved to normal agricultural land (Table 1).

Table 1 Physical and Chemical properties of soil of experimental site

| | Results | |
|---|---|---|
| Tests | Zero time | After six months |
| Texture | Black | Brown |
| Bulk density | 0.99 gm/cm$^3$ | 1.2 gm/ cm$^3$ |
| Water holding capacity | 52% | 65% |
| PH | 7.77 | 7.65 |
| Organic carbon | 4.8% | 2.6% |
| Total nitrogen | 0.08% | 0.09% |
| Potassium | 175 ppm | 165 ppm |
| Available phosphorus | 62 ppm | 72 ppm |

## Composition of oily sludge

The composition of oily sludge which was lying in tank dyke of crude tank 115 was analysed. Oily sludge on extraction shows solvent extractable TPH 78%, sediments 9% and 13% water content (Table 2). The extractable TPH obtained from oily sludge was further fractionated into various fractions showed 67% alkanes, 20% aromatics, 11% asphaltenes and 2% NSO (nitrogen, oxygen, sulphur containing compounds) as shown in Table 3.

**Table 2** Characterization of oily sludge of crude tank 115

| Composition of oily sludge | Results |
|---|---|
| Total petroleum hydrocarbon (Solvent extractable) | 78% |
| Sediments/Ash | 9% |
| Water | 13% |

**Table 3** The composition of total petroleum hydrocarbon extracted from oily sludge of crude tank No.115

| Fractions | Percent (%) |
|---|---|
| Alkane (Saturated fraction) | 67% |
| Aromatic | 20% |
| Asphaltane | 11% |
| NSO | 2% |

## Bioremediation of oily sludge

Oily sludge contamination levels in soil of treatment blocks of B, C and D are shown in Figure 3. Block C and D were treated with Oilzapper plus nutrient, while Block C was maintained as control which was treated with nutrient alone.

At zero time (just before initiation of work), the oily sludge (TPH) contamination in block B was 26.5%. With application of Oilzapper plus nutrient, the oily sludge (TPH) contamination reduced from 26.5% to 1.2% in six months which revealed 95.4% biodegradation of oily sludge in six months. With application of Oilzapper and nutrients in block B, the oily sludge (TPH) contamination in soil of block B after two and four months was 15.9% and 5.1% respectively showing biodegradation of 40% and 80.7% respectively (Figure 3). Similarly, in block D, the oily sludge concentration recorded was 31.0% at zero time. With application of Oilzapper and nutrient, the sludge concentration reduced from 31.0% to 1.8% which accounted for 94.1% biodegradation of sludge in six months. The oily sludge contamination (solvent extractable TPH)

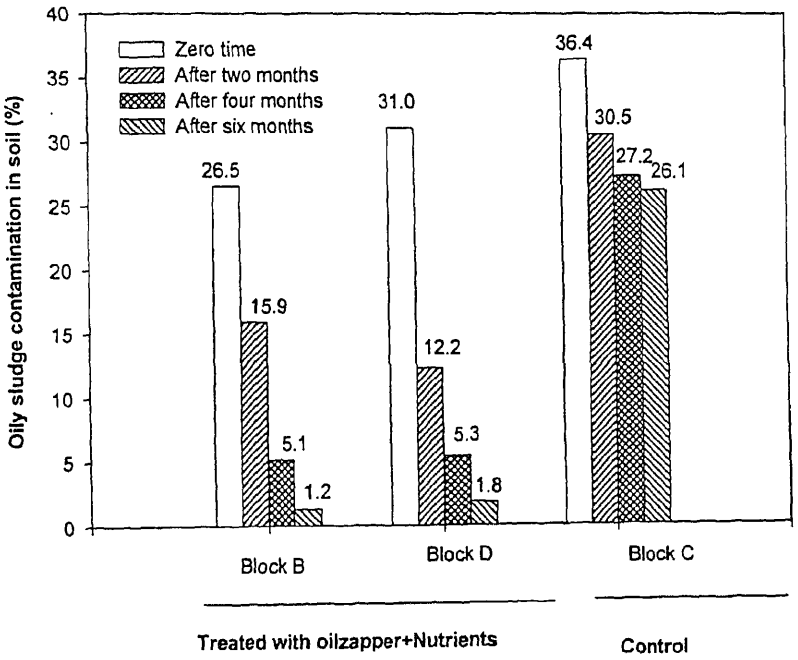
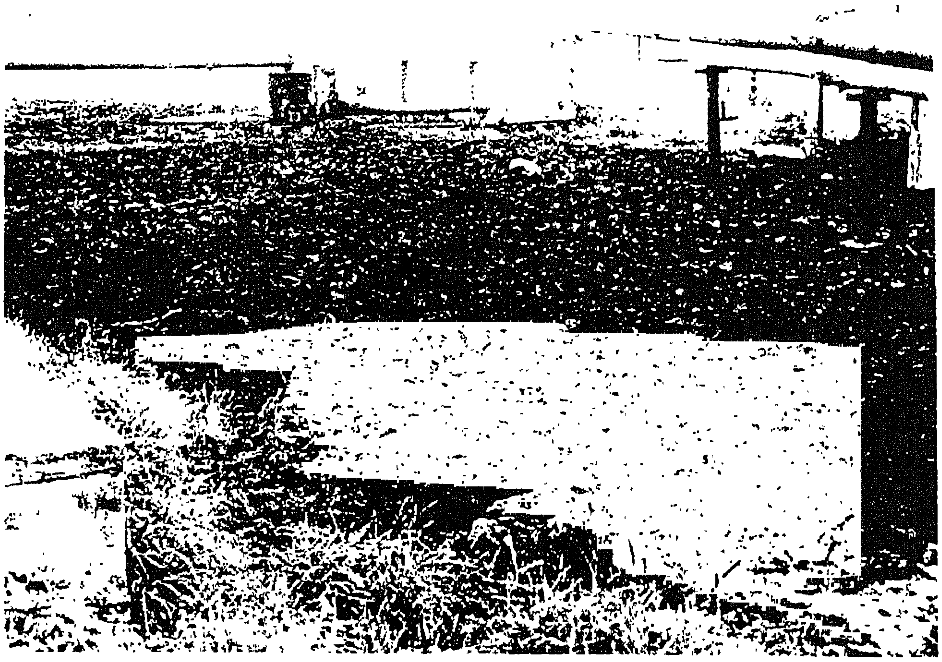
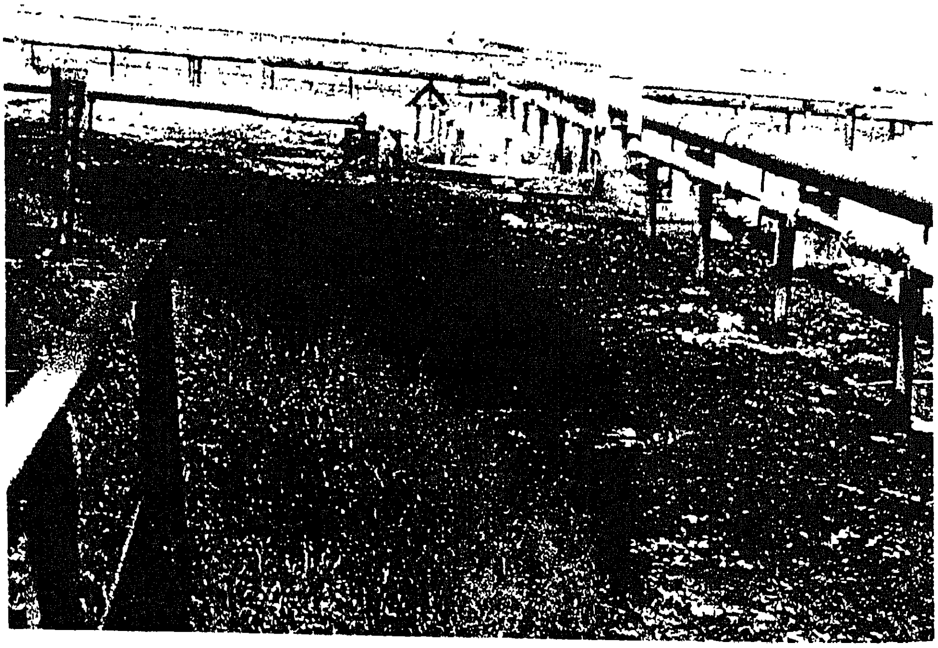


Figure 3. Bioremediation of oily sludge in tank dyke of tank 115

in soil of block D at bioremediation site after two months and four months of initiation of bioremediation was 12.2% and 5.3% revealing biodegradation of 60.6% and 82.9% respectively (Figure 3). However, block C was maintained as control (treated only with nutrients) showed the oily sludge contamination reduced from 36.4% to 26.1% in six months which accounted for only 28.2% biodegradation of sludge in control block. The sludge contamination in soil of control block C (treated with nutrients alone) after two and four months was 30.5% and 27.2% showing a loss of 16.2% and 25.2% respectively which may be due to indigenous microorganisms present at bioremediation site (Figure 3). Bioremediation site before bioremediation and after two moths of biodegradation of oily sludge is shown in Figure 4. As shown in Figure 5, oily sludge was converted into brown soil after biodegradation of total petroleum hydrocarbon by Oilzapper. The sludge toxicity has also reduced after biodegradation of oily sludge (Figure 6).



Before bioremediation of oily sludge



After two months of bioremediation of oily sludge

**Figure 4.** Oily sludge bioremediation site in tank farm area of tank 115

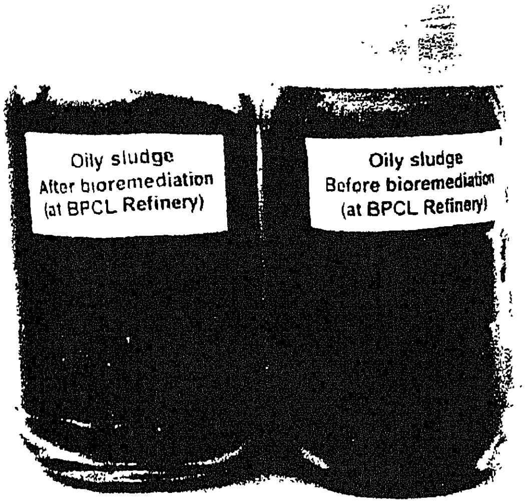
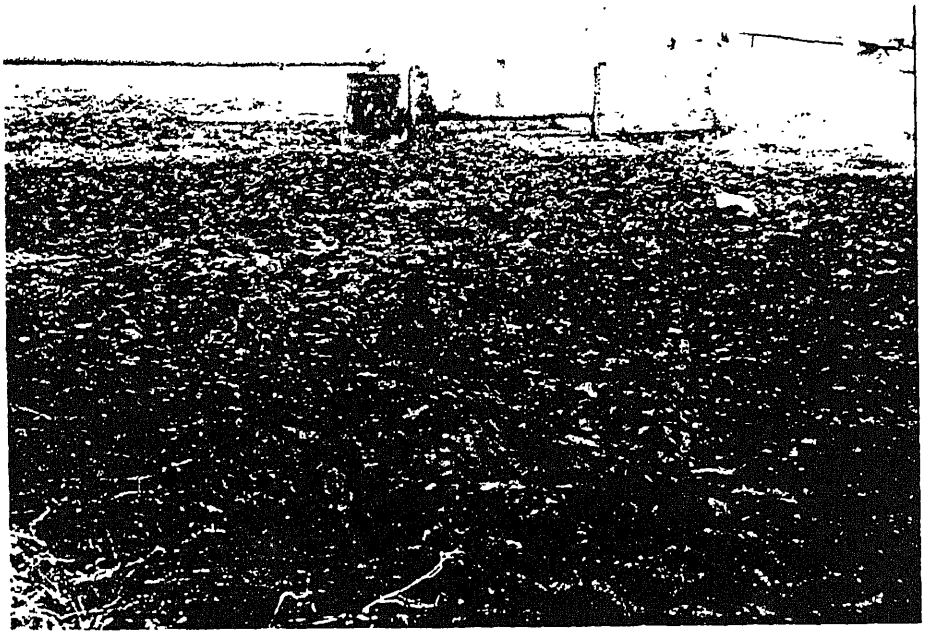


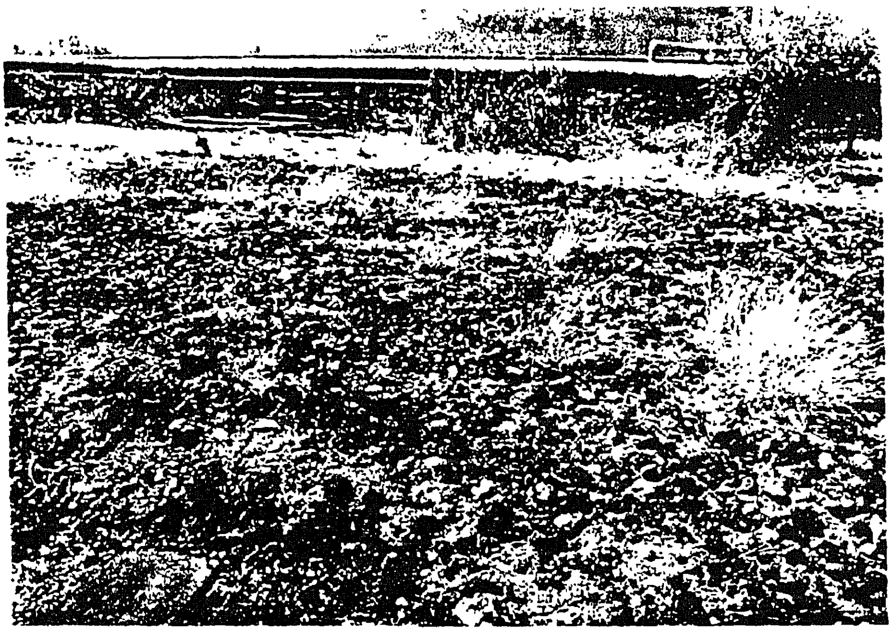
**Figure 5.** Bioremediation of tank bottom sludge by Oilzapper - an oily sludge degrading bacterial system

## *Bioremediation of various fractions of oily sludge*

Bioremediation of various fractions of oily sludge at bioremediation site was estimated. Block B in tank dyke of tank 115 was treated with Oilzapper (oily sludge degrading bacteria) plus nutrients showed 95.4% biodegradation of oily sludge in six months of which alkane fraction was accounted for 66.1%, aromatic fraction 19.4% and NSO plus asphaltane fraction 9.9% (Table 4). Similarly, biodegradation of various fractions of oily sludge in two months and in four months is also shown in Table 4. GC fingerprinting of biodegradation of alkane and aromatic fraction in block B is shown in Figure 7 and 8 respectively. Block D was also treated with Oilzapper plus nutrients revealed 94.1% biodegradation of oily sludge (TPH) in six months. Out of 94.1% biodegradation of oily sludge, the biodegradation of alkane, aromatic and NSO plus asphaltane fraction was accounted for 66.4%, 19.2% and 8.5% respectively (Table 5). GC fingerprinting of alkane and aromatic fraction in block D is shown in Figure 9 and 10 respectively.



Before bioremediation of oily sludge



After bioremediation of site

(toxicity of sludge has reduced and grasses sprouted in treated site)

**Figure 6.** Bioremediation of oily sludge and reduction of sludge toxicity

Table 4 Biodegradation of various fractions of oily sludge in block B at bioremediation site in tank dyke of tank 115

| | Bioremediation (%) | | |
|---|---|---|---|
| Oily sludge fraction | In two months | In four months | In six months |
| Total petroleum hydrocarbon | 40.0 | 80.7 | 95.4 |
| Alkane fraction | 26.5 | 56.3 | 66.1 |
| Aromatic fraction | 11.6 | 19.1 | 19.4 |
| NSO + Asphaltane fraction | 1.9 | 5.3 | 9.9 |

Table 5 Biodegradation of various fraction of oily sludge in block D at bioremediation site in tank dyke of tank 115

| | Bioremediation (%) | | |
|---|---|---|---|
| Oily sludge fraction | In two months | In four months | In six months |
| Total petroleum hydrocarbon | 60.6 | 82.9 | 94.1 |
| Alkane fraction | 44.8 | 58.7 | 66.4 |
| Aromatic fraction | 12.5 | 15.6 | 19.2 |
| NSO + Asphaltane fraction | 3.3 | 8.6 | 8.5 |

Block C was treated only with nutrients alone exhibited only 28.2% biodegradation of oily sludge in six months. In block C, biodegradation of alkane, aromatic, NSO plus asphaltane fraction in 2, 4 and 6 months were also less (Table 6) as compared to biodegradation of these fractions in block B and D which were treated with Oilzapper plus nutrients. GC fingerprinting of biodegradation of alkane and aromatic fractions in block C are shown in Figure 11 and 12 respectively.

Table 6 Biodegradation of various fractions of oily sludge in block C at bioremediation site in tank dyke of tank 115

| | Bioremediation (%) | | |
|---|---|---|---|
| Oily sludge fraction | In two months | In four months | In six months |
| Total petroleum hydrocarbon | 16.2 | 25.3 | 28.2 |
| Alkane fraction | 8.3 | 16.8 | 18.4 |
| Aromatic fraction | 6.9 | 7.5 | 8.1 |
| NSO + Asphaltane fraction | 1.0 | 1.0 | 1.7 |

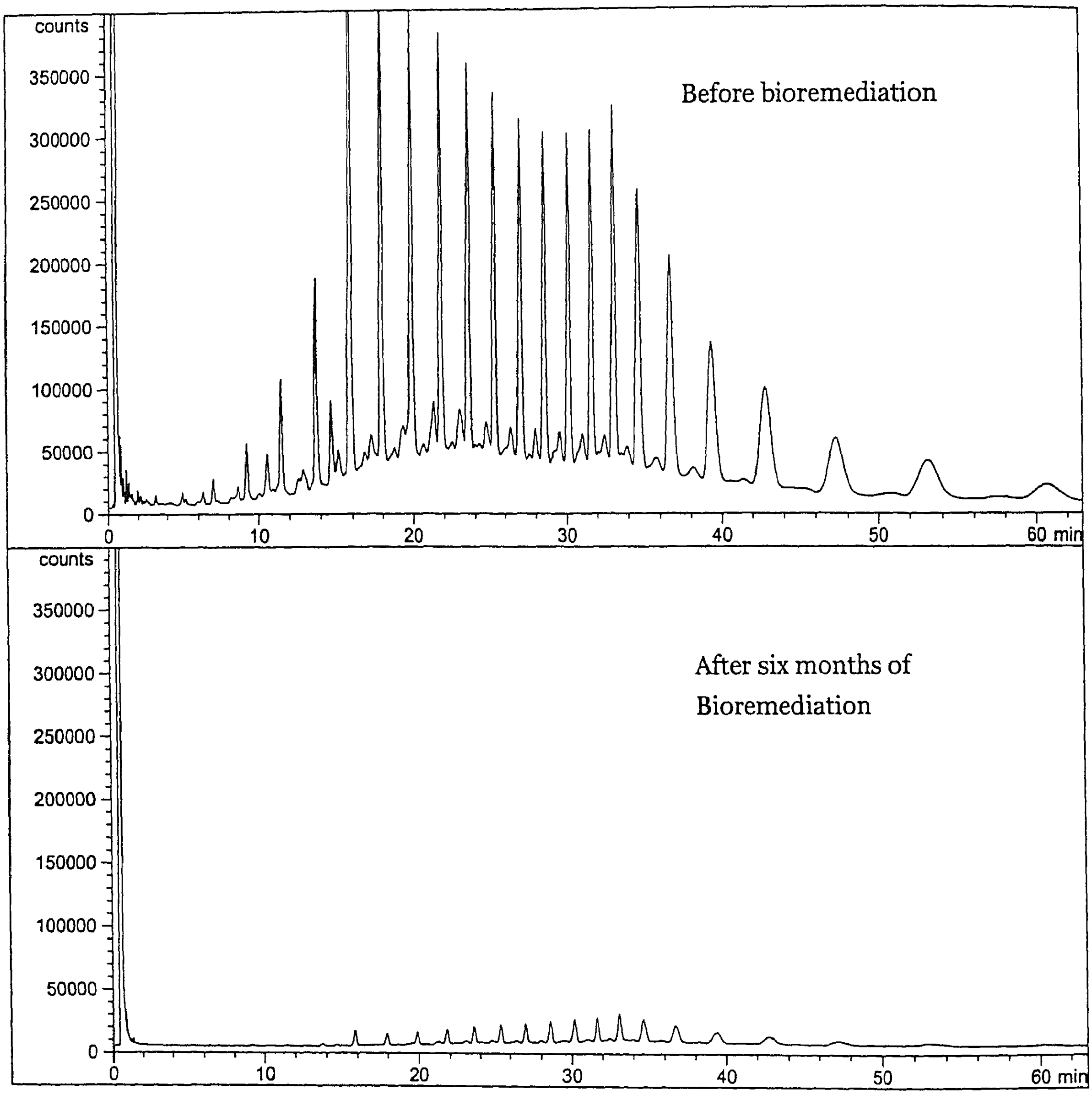


**Figure 7.** GLC finger printing of alkane fraction of oily sludge from treatment block B, treated with Oilzapper plus nutrients

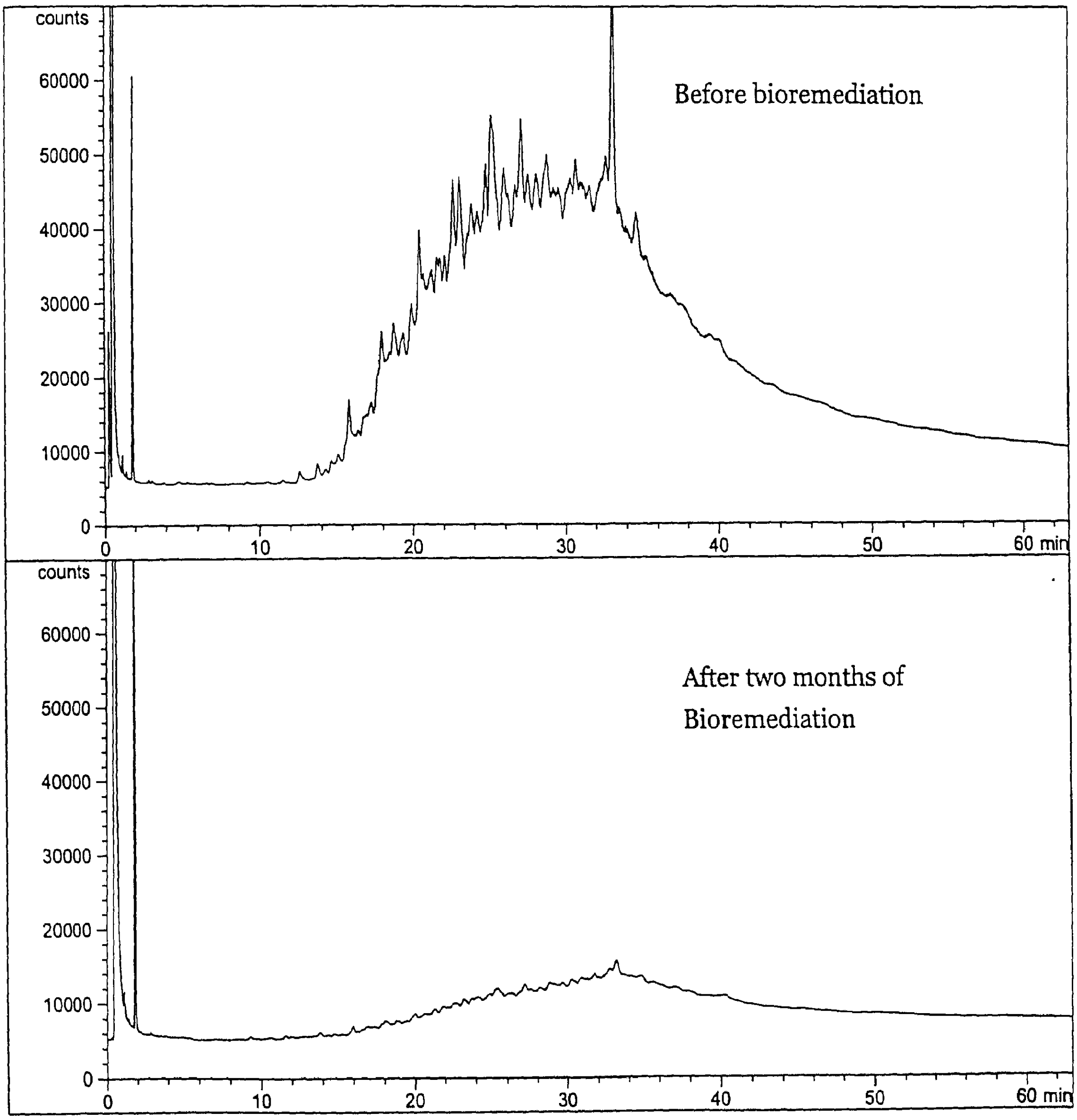
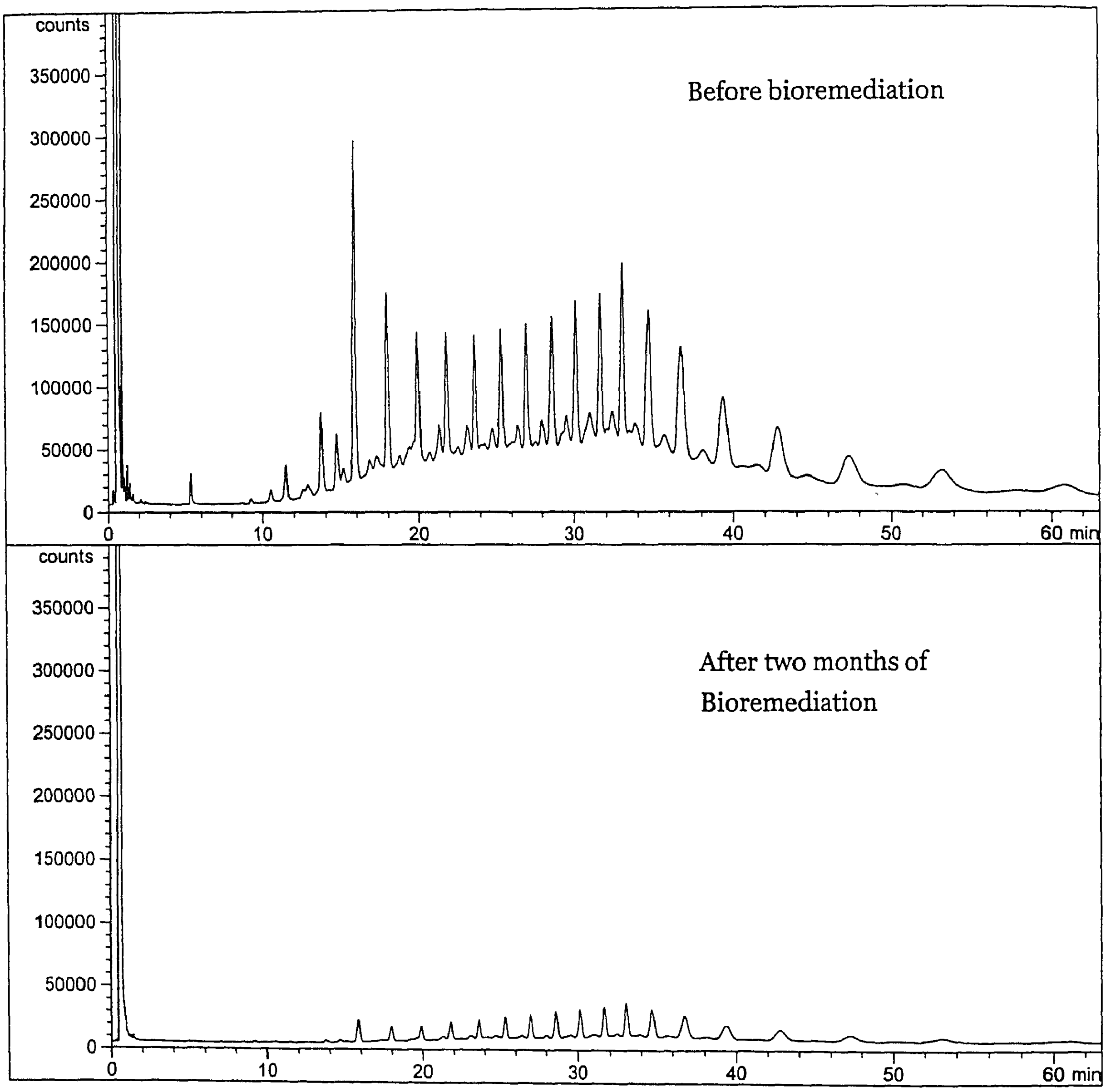


**Figure 8.** GLC finger printing of aromatic fraction of oily sludge from treatment block B treated with Oilzapper plus nutrients



Before bioremediation

After two months of Bioremediation

**Figure 9.** GLC finger printing of alkane fraction of oily sludge from block D treated with Oilzapper plus nutrients

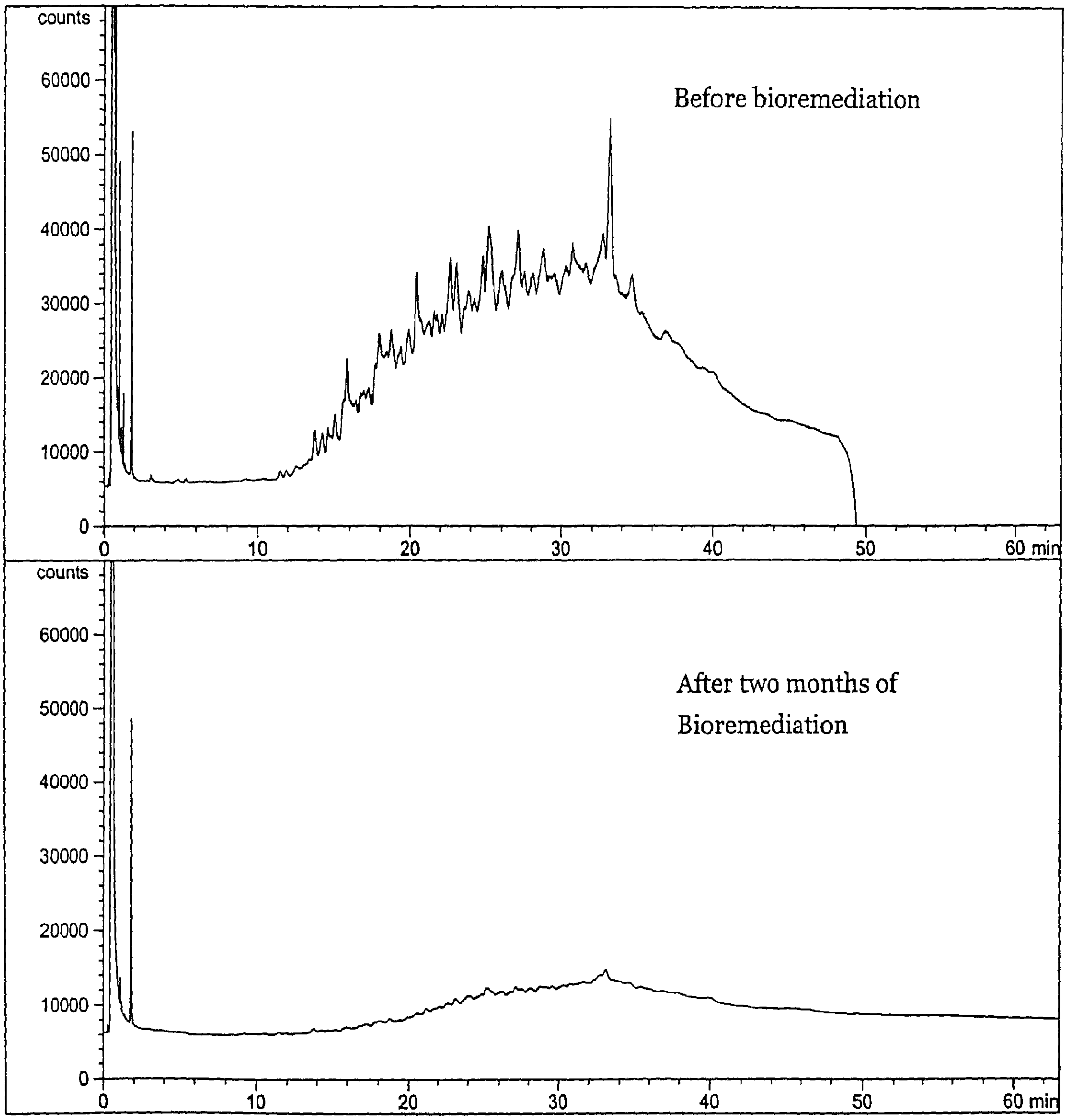


**Figure 10.** GLC finger printing of aromatic fraction of oily sludge from treatment block D treated with Oilzapper plus nutrients

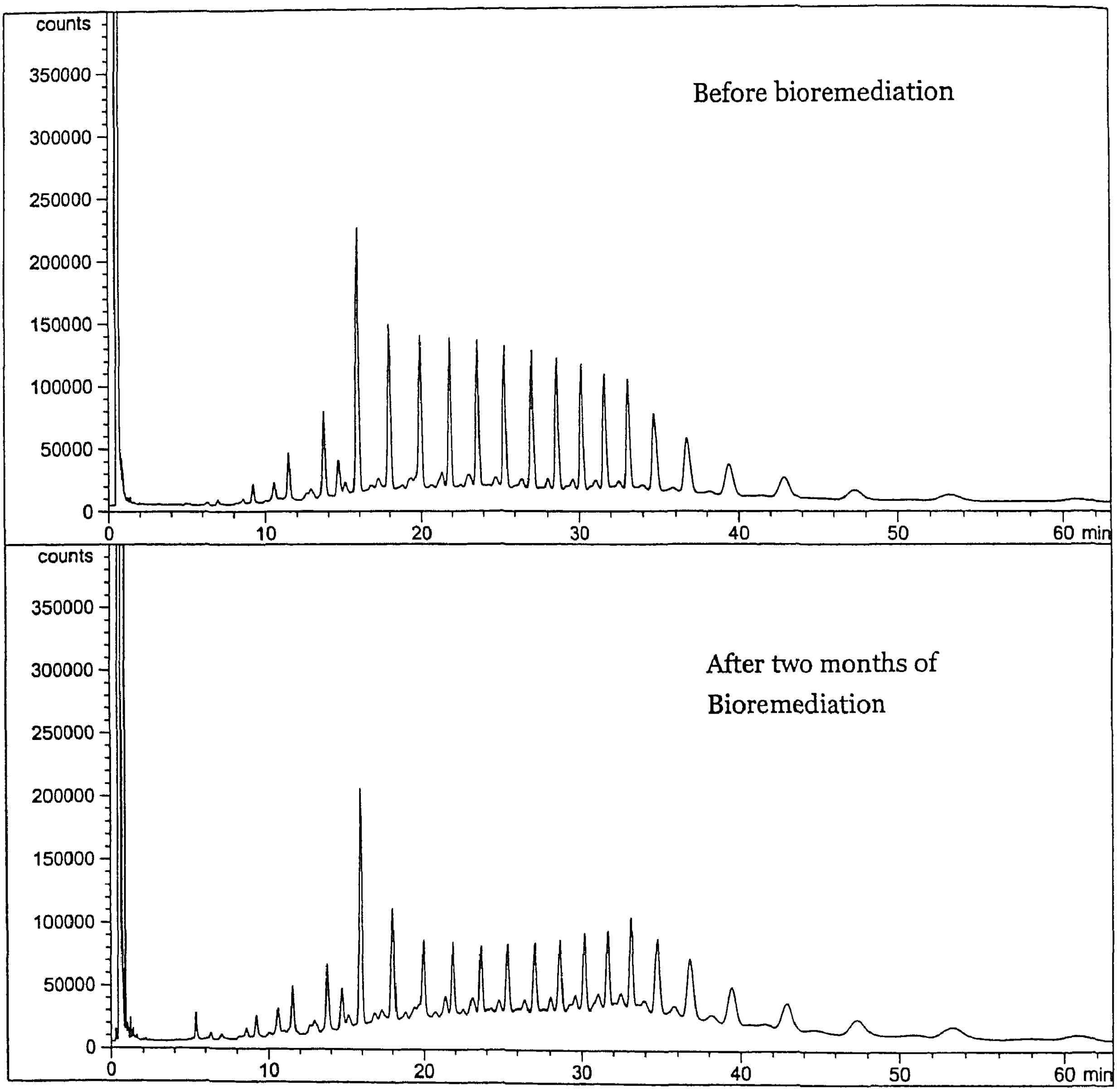


**Figure 11.** GLC finger printing of alkane fraction of oily sludge from control block C, treated with nutrients alone

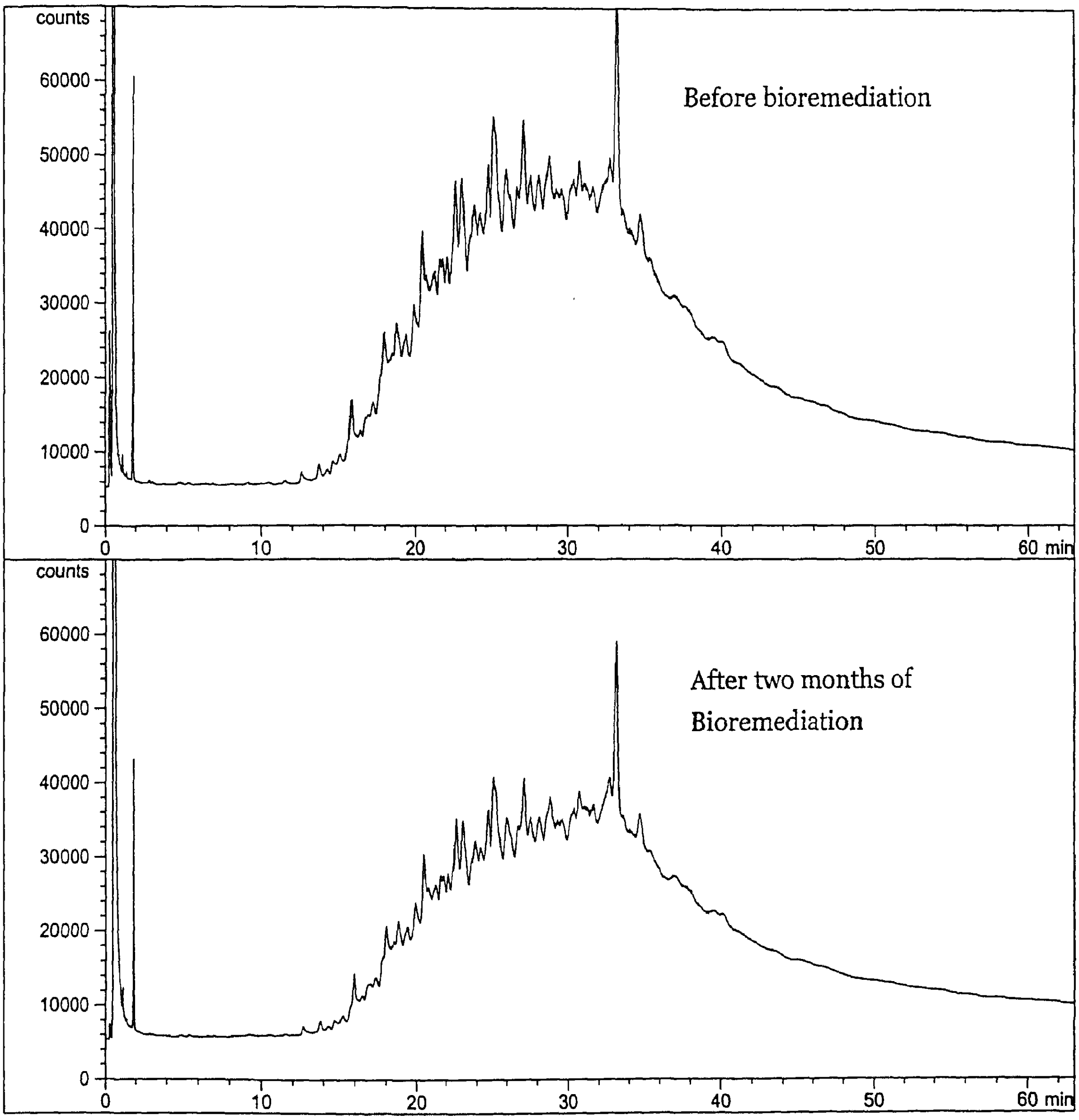


**Figure 12.** GLC finger printing of aromatic fraction of oily sludge from control block C, treated with nutrients alone

# 4 Conclusion

- The experiment on bioremediation of oily sludge by oily sludge degrading bacterial system Oilzapper was initiated in the month of October 1999 in the tank dyke of crude tank 115 at BPCL refinery.
- Bioremediation site (in tank farm area) was divided into block B, C and D. Blocks B and D were treated with Oilzapper plus nutrient, while block C was treated only with nutrient.
- Block B was treated with Oilzapper plus nutrients showed that oily sludge in soil of tank farm area reduced from 26.5% to 1.2% in six months which revealed 95.4% biodegradation of oily sludge in six months.
- Block D was also treated with Oilzapper plus nutrients exhibited that the oily sludge concentration reduced from 31.0% to 1.8% in six months which indicated 94.1% biodegradation of sludge in six months.
- In control block C, oily sludge concentration reduced from 36.4% to 26.1% in six months which resulted in only 28.2% biodegradation of sludge in six months.
- In block B, more than 98% alkane fraction and aromatic fraction of oily sludge have been biodegraded by Oilzapper in six months. Similarly, in block D, more than 96% alkane fraction and aromatic fractions of oily sludge have been biodegraded in six months. However, in untreated control block, only 29% of alkane fraction and only 40% of aromatic fraction of oily sludge have been biodegraded in six months.